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A NEW NORSESTERTERPENOID FROM THE SPONGE SPECIES *Sarcotragus*

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*A new norsesterterpenoid **1** was isolated from the* Sarcotragus *sponge by bioactivity-guided fractionation. The structure was established on the basis of NMR and MS analysis.*

Keywords: *Sarcotragus*, marine sponge, norsesterterpenoid.

Marine sponges of the order Dictyoceratida are known to contain various furanosesterterpene derivatives that possess interesting biological properties [1, 2]. Dozens of new furano- and pyrroloterpenoids [3–8], three cyclitol derivatives [9], lipids [10, 11], indole alkaloids [12], and a trisoxazole macrolide [13] have been isolated from *Sarcotragus* sp. (Dictyoceratida) collected from Korean waters. The *Sarcotragus* genus showed diverse bioactivities, such as inhibiting NF-kappa B signaling [14], inducing cell cycle arrest and apoptosis [15], and cytotoxic activity [5]. In a continuing study, a new norsesterterpenoid **1** was isolated from the same sponge. The gross structure of the compound was elucidated by the aid of COSY, HMQC, and HMBC experiments. The isolation and structure elucidation of the new compound are described herein.

The methanol extract of the sponge displayed cytotoxicities against five human tumor cell lines (see Experimental) and showed toxicity to brine shrimp larvae (LD_{50}, 93 μg/mL). Guided by the brine shrimp assay, the methanol extract was successively fractionated employing reversed-phase flash column chromatography and ODS HPLC to afford compound **1** as a new bioactive component.

The sponge was collected in July 1998 (15–25 m depth), off Cheju Island, Korea. This specimen was identified as *Sarcotragus* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen (J98J-5) of the sponge (registry No. Por. 33) was deposited in the Natural History Museum, Hannam University, Taejon, Korea, and has been described elsewhere [3].

Sarcotin P (**1**) was isolated as a light yellow oil. The molecular formula of **1** was established as $C_{24}H_{36}O_4$ on the basis of HR-FAB-MS. The presence of the furan moiety and the 1,1,4-trisubstituted diene were established by analysis of NMR data as well as by comparison of the data with that of sarcotin A [3]. A trisubstituted olefine (δ 5.28, H-17) and a 1,1,4-trisubstituted diene (δ 6.18, H-11; 5.77, H-10; 5.37, H-12) were found, while the geometry of the disubstituted double bond (C-11) was determined to be *E* based on the coupling constant of the respective protons (J = 15.0 Hz). The downfield carbon chemical shift of the C-19 methyl (δ_C 24.3) indicated the *Z* geometry of this trisubstituted double bond, which was also supported by the upfield shift of the C-20 signal (δ_C 35.9) compared to that of palinurin (δ_C 41.6, C-20) [16]. A 2D COSY analysis allowed correlation from the trisubstituted double bond through an allylic methylene to the positioning of the trisubstituted double bond. Most of the ^1H and ^{13}C NMR spectra of **1** were in accordance with those of sarcotin A [3]. However, the signals corresponding to the nonconjugated tetronic acid terminus were replaced by the substructure α,β-dihydroxy-γ-butanone moiety. This partial structure was the same as the previous isolated sacotin O, sacotrine F, and isosarcotrine F [5]. Signals of two oxymethine (δ 4.06, δ_C 72.0; δ 3.95, δ_C 80.1) and a methyl ketone signal (δ 2.16, δ_C 26.8, and δ_C 214.3) were observed. HMBC correlations were observed among allylic methylene (δ 2.42, dd, J = 14.0, 6.5 Hz and δ 2.28, dd, J = 14.0, 7.0 Hz) coupling to δ_C 129.5, 132.5, 72.0, and 80.1. The key COSY and HMBC correlations are shown in Fig. 1.

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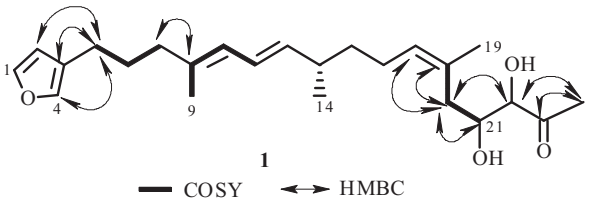

Fig. 1. Key HMBC and COSY correlations of **1**.

The configuration at C-13 was assumed to be the same as that of sarcotin A [4]. An attempt to convert **1** to its MTPA esters by the modified Mosher′s method was unsuccessful due to the rapid decomposition of the reactant.

EXPERIMENTAL

Extraction, Isolation, and Activities. The frozen sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract of the sponge displayed moderate cytotoxicities against five human tumor cell lines (ED_{50} values for A549, SK-OV-3, SK-MEL-2, XF498, and HCT15 were 19.0, 20.3, 11.8, 15.5, and 12.6 μg/mL, respectively). The MeOH extract was partitioned between water and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between 90% methanol and *n*-hexane to yield 90% methanol (54 g) and *n*-hexane soluble (13 g) fractions. As described in our previous report [2], the 90% methanol fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with a solvent system of 25 to 0% H_2O–MeOH, to afford 20 fractions (Fr.1–Fr.20). These fractions were evaluated for activity in the brine shrimp assay, and Fr.6–Fr.9 were found to be active. These fractions were combined (7.4 g) and further separated by reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with 25 to 0% H_2O/MeOH, to afford ten fractions. Fractions 6-5–6-7 (5.4 g) were combined, and the combined fraction was further separated by reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with 33 to 0% H_2O/MeCN, to afford 13 fractions. Guided by the brine shrimp assay, compound **1** (1.3 mg) was obtained by purification of fraction Fr.6-5 by ODS HPLC.

Sarcotin P (1). Light yellow oil. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.37 (1H, br.s, H-1), 6.29 (1H, br.s, H-2), 7.25 (1H, br.s, H-4), 2.39 (2H, t, J = 7.0, H-5), 1.66 (2H, m, H-6), 2.07 (2H, t, J = 7.5, H-7), 1.72 (3H, s, H-9), 5.76 (1H, d, J = 11.0, H-10), 6.17 (1H, dd, J = 15.0, 11.0, H-11), 5.40 (1H, dd, J = 15.0, 8.5, H-12), 2.15 (1H, m, H-13), 1.01 (3H, d, J = 7.0, H-14), 1.34 (2H, m, H-15), 2.03 (2H, m, H-16), 5.27 (1H, t, J = 7.0, H-17), 1.76 (3H, s, H-19), 2.42 (1H, dd, J = 14.0, 6.5, H-20a), 2.22 (1H, dd, J = 14.0, 7.0, H-20b), 4.06 (1H, td, J = 6.5, 2.0, H-21), 3.95 (1H, d, J = 2.0, H-22), 2.16 (3H, s, H-23). ^{13}C NMR (150 MHz, CD_3OD, δ): 143.7 (C-1), 112.0 (C-2), 126.4 (C-3), 140.1 (C-4), 24.8 (C-5), 29.5 (C-6), 40.3 (C-7), 136.6 (C-8), 16.5 (C-9), 126.4 (C-10), 126.6 (C-11), 139.1 (C-12), 38.0 (C-13), 21.4 (C-14), 38.4 (C-15), 26.9 (C-16), 129.5 (C-17), 132.5 (C-18), 24.3 (C-19), 35.9 (C-20), 72.0 (C-21), 80.1 (C-22), 214.3 (C-23), 26.8 (C-24). FAB-MS *m/z* 410 $[M + Na]^+$ (3), 388 (8); HR-FAB-MS *m/z* 410.2501 (calcd for $C_{24}H_{36}O_4Na$, 410.2512).

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